STUDIES ON LIVING PROTOPLASM

II. THE PHYSICAL STRUCTURE OF THE NUCLEUS OF THE ECHINODERM OOCYTE

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(With Three Text-figures)

INTRODUCTION

By observation of the oocyte of *Echinus esculentus* through a horizontal microscope, Gray (1927) showed that the nucleolus could fall through the fluid contents of the germinal vesicle under the action of gravitational force. He estimated the velocity of fall as $0.4 \mu$ per sec. The present writer has attempted to gain some information about the physical state of the nucleus by employing this velocity of fall as an indicator of the viscosity of the nuclear protoplasm. In the course of experiments commenced in 1932 to investigate the effect of temperature changes on the viscosity, certain very peculiar results were obtained. It is proposed to confine the present paper to a description of these results, and to consider the effect of temperature on nuclear viscosity in a later paper.

The experimental method does not permit the determination of the absolute coefficient of viscosity of the nuclear protoplasm, since the specific gravities of the nucleolus and nuclear fluid are unknown. It might be possible to obtain an approximate value for the density of the nucleus as a whole by dissecting it out of the oocyte and determining its rate of fall in a solution of known viscosity. However, Chambers (1924) has pointed out that the isolation of the nucleus in such oocytes is usually followed by profound changes in its consistency, and results obtained for the density of such an isolated nucleus would probably have little value.

In any event, the difficulty of obtaining an estimate of the specific gravity of the nucleolus appears insuperable. The nucleolus usually disappears on puncturing the nuclear membrane of the oocyte—sometimes, indeed, even on injuring the cytoplasm by the insertion of a microneedle; complete isolation of the nucleolus seems impossible. Heilbrunn (1928) has deduced a value for the absolute viscosity of the nuclear fluid by estimating the densities on theoretical grounds; his calculation will be referred to in a later part of the present paper.

It is necessary first to consider the extent to which the velocity of fall gives a true
estimate of the nuclear viscosity. If the viscous drag on the nucleolus is equal to that predicted from Stokes's law, the motion will be determined by the equation

$$\frac{4\pi}{3} a^3 (\sigma - \rho) g - 6\pi \eta a v = \frac{4\pi}{3} a^3 \sigma \frac{dv}{dt},$$

......(1)

which, becomes, after integration,

$$v = \frac{2a^2 (\sigma - \rho) g}{9\eta} \left(1 - \exp \left[1 - \frac{9\eta t}{2a^2 \sigma}\right]\right),$$

......(2)

where $v$ = velocity of the falling sphere at time $t$, 
$a$, $\sigma$ = the radius and density of the falling sphere
$\rho$ = the density and absolute viscosity coefficient of the surrounding fluid,
$g$ = the acceleration due to gravity.

After an infinitely long time a terminal velocity, $\bar{V}$, is attained, having a value

$$\bar{V} = \frac{2a^2 (\sigma - \rho) g}{9\eta}.$$  

......(3)

Equation (2) can therefore be expressed in terms of the terminal velocity as

$$v = \bar{V} \left(1 - \exp \left[- \frac{9\eta t}{2a^2 \sigma}\right]\right).$$

......(4)

If now in equation (4) we substitute the values experimentally determined, 
$a = 10^{-3}$ cm., the nucleolar radius,
$\eta = 0.1$, an approximate value for the absolute viscosity of the nuclear fluid, obtained by an independent experimental method.
$\sigma = 1.14$, Heilbrunn's estimate for the density of the nucleolus, we have

$$v = \bar{V} (1 - e^{2.5 \times 10^{-5}}).$$

......(5)

The exponential term in the bracket is less than 0.01 when $t$ is greater than $10^{-5}$ sec., and is less than 0.001 when $t$ exceeds $2 \times 10^{-5}$. Hence the terminal velocity of fall is attained, within 1%, in approximately $10^{-6}$ sec. and within 0.1% in about twice this time. (This somewhat surprising result does not indicate a high acceleration, since the terminal velocity is very small.)

The above treatment is only an approximation, since it assumes an infinite volume of nuclear fluid, but it is sufficiently accurate to show that in any system such as the oocyte nucleus the terminal velocity of the nucleolus is attained almost instantaneously. It is therefore safe to assume that the velocity of the nucleolus at any instant during its fall does not differ appreciably from the terminal velocity characteristic of the conditions under which it is falling.

We may therefore write

$$v = \frac{ck}{\eta},$$
where \( v \) = the instantaneous velocity of fall of the nucleolus,

\[
\epsilon = \frac{2a^3(a - \rho)}{9}
\]

and is a constant for any single oocyte,

\( k = \) the Ladenburg constant.

The Ladenburg constant is introduced when, as in the present instance, the movement takes place in a restricted volume of fluid. If the fluid is enclosed in a cylindrical container, and the moving sphere traverses the axis of the cylinder, it has been shown that

\[
k = \frac{1}{\left(1 + \frac{a}{R}\right)\left(1 + \frac{a}{L}\right)}
\]

where \( a = \) the radius of the moving sphere,

\( R, L = \) the radius and length, respectively, of the confining cylinder.

If, as in the nucleus, the boundary of the fluid be spherical instead of cylindrical, the value of the Ladenburg constant varies with the position of the nucleolus. The maximum value of \( k \), corresponding to the most rapid rate of fall, will occur when the nucleolus is approximately concentric with the nuclear boundary. An experimental determination of \( k \) under these conditions (referred to in a later part of this paper) gave a maximum value of 0.29 when two such spheres are concentric.

The curve in Fig. 1 shows the relationship between the distance fallen and the time in a single experiment with the oocyte of *Echinus esculentus*. At the commencement and at the end of the fall, the nucleolus is almost in contact with the nuclear membrane. The velocity of fall is at first very low; it increases to a maximum, remaining appreciably constant at this value for some time, and finally decreases once more as the nuclear boundary is approached (see also Fig. 3, which is a graph of velocity against time for the same observation). The constant maximum velocity attained is the value used throughout the present work as an indicator of the viscosity of the nuclear fluid. It is to be noted that the relationship between this velocity and the viscosity of the nuclear fluid will only be constant for a single oocyte, since the nucleolar radius, the value of the Ladenburg constant, and the densities of the nucleolus and fluid need not be the same for different oocytes.

**MATERIAL AND METHODS**

For a preliminary examination, the method of mounting the oocytes used by Gray (1927) was employed. A glass slip was ringed with vaseline; a small piece of well-teased cotton-wool was placed in the centre of the vaseline ring, and a drop of sea water containing the oocytes was placed on the cotton-wool centre. A cover-glass was then applied to the vaseline and pressed down until the oocytes were held very lightly but firmly in the meshes of the cotton-wool. The slide was then mounted on the rotating stage of a suitable microscope tilted into the horizontal position.

No fall of the nucleolus was observed in oocytes of the following species: *Perinereis cultrifera*, *Sabellaria alveolata*, *Branchiomma vesiculosum*, *Pomatoceros*
triqueter, Lumbricus terrestris, Teredo navalis, Periplaneta americana, Holothuria forskali. In five species of echinoderms, however, in Marthasterias glacialis, Luidia ciliaris, Echinus esculentus, Psammechinus miliaris, and Echinocardium cordatum a fall of the nucleolus was clearly seen. Throughout the present work, the results dealt with are those obtained on oocytes of Echinus esculentus, but the principal conclusions have been confirmed on a few experiments on oocytes of Psammechinus miliaris and Marthasterias glacialis.

As the experiments had originally been undertaken to determine the effect of temperature variation on the viscosity, a special cell was designed to contain the oocytes. In order to facilitate rapid heating and cooling, the cell was made of brass, subsequently gold plated to eliminate toxic effects of the metal. The cell, which was the size of a normal microscope slide, contained a circular window, and the oocytes were held in this central space between two cover-slips; a pair of minute varnished copper-constantan thermocouples cemented into the central cavity served to indicate the temperature during the experiments. The first determinations were performed with a modification of this cell which permitted the constant irrigation of the oocytes with oxygenated sea water. It was found, however, that the phenomena observed were quite independent of the irrigation if the experiment did not last longer than about an hour. Since the difficulties of irrigating such a small apparatus were considerable, about half the present experiments were carried out without this refinement.

In order to record the motion of the nucleolus as accurately as possible, the fall was photographed by a slow-running cinematograph camera attached to the microscope. The photographic frequency was approximately 8 frames per minute, and since a single fall occupied 3–4 min., this frequency was adequate to obtain perfectly sharp images of the nucleolus. A few of the experiments described have been carried out by visual observation, the rate of fall being determined with the aid of a stopwatch and micrometer eyepiece. But in spite of the considerable labour involved (approximately 20,000 measurements have been made on enlargements from the cinema film in the present work) the photographic method is far superior to the visual, since it eliminates completely the subjective element, and in addition records all movements (e.g. lateral and rotational) of the nucleolus during its fall.

The apparatus having been set up with a suitable oocyte in the field of the microscope, a few minutes were allowed to elapse in order that the nucleolus should come to rest at the lowest point of the nucleus. The stage was then rotated through 180°, and the fall of the nucleolus recorded. The stage was then rotated back to the original position, and the nucleolus was thus made to traverse its previous path, but in the opposite direction. The fall could be repeated as many times as desired.

RESULTS
A. Homogeneity of the nuclear fluid

A typical distance-time graph for a single fall of the nucleolus is shown in Fig. 1. The fall is perfectly smooth, there being no sudden acceleration or stoppage in the motion. It is evident that there exist no large heterogeneities in the nuclear fluid.
Photographs taken at the highest possible magnification with higher recording frequencies have failed to detect any irregularity in the motion. These observations do not exclude the possibility that the fluid may show graded viscosity differences between different regions of the nucleus, but evidence will be presented later to indicate that such regional viscosity differences do not exist; the nuclear protoplasm appears to be a homogeneous fluid.

![Graph showing the relation between distance and time for a single fall of the nucleolus.](image)

**Fig. 1.** Relation between distance and time for a single fall of the nucleolus. Ordinates: distance travelled in μ; abscissae: time in minutes.

**B. Thixotropic properties of the nuclear fluid**

In a series of successive falls, the maximum velocity attained during the initial fall is significantly less than that of later falls. The second fall of such a series, however, does not differ appreciably from the subsequent falls. Table I presents the

<table>
<thead>
<tr>
<th>Velocity of...</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial fall</td>
<td>0.86</td>
<td>0.90</td>
<td>0.68</td>
<td>0.68</td>
<td>0.78</td>
<td>0.86</td>
<td>0.65</td>
<td>0.77</td>
</tr>
<tr>
<td>Second fall</td>
<td>1.08</td>
<td>1.00</td>
<td>0.80</td>
<td>1.04</td>
<td>1.01</td>
<td>1.11</td>
<td>0.93</td>
<td>0.99</td>
</tr>
<tr>
<td>Mean of later falls</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>
results of seven observations on different oocytes, the values for the velocities being calculated with reference to the mean value of the third and later falls; this mean value is taken as unity. (This is necessary, since different oocytes may vary as much as 20% in their mean velocity of fall.)

At any time after the commencement of such an experiment, it is possible to reproduce the original slow fall by allowing the oocyte to stand for 10 min.; the reconstitution of the elastic structure of the nucleus takes place with considerable rapidity. The previous mechanical history of the oocytes was not carefully controlled; it is probable that some of the variation in the value of the velocity of the first fall shown in the table is due to different resting periods before the commencement of the experiment. On the other hand, the variation in the value of the velocity during the second fall is due to the "polarity" phenomenon described in the next section of the paper. It is sufficient here to point out that the mean value of the velocity during this second fall is not significantly different from that of later falls.

In addition to demonstrating that the nuclear protoplasm possesses a definite structural elasticity in the resting state, these results throw some light on present controversies with respect to the elasticity of protoplasm. Seifriz (1924), by measuring the effect of a magnetic field on the movement of a nickel sphere embedded in the protoplasm of the egg of *Echinarchnus parma*, deduced that the cortical protoplasm was elastic. His experiment indicated an elastic yield point below which a displacement of the sphere was followed by its return to the original position when the moving force was switched off. Two years previously, A. Heilbronn (1922) had reported the result of the orientation of iron filings suspended in the protoplasm of various plasmodia subjected to a magnetic field. He had failed to find an elastic yield point, but his experiments indicated a decrease in the protoplasmic viscosity during repeated reorientations. Both these experiments have been severely criticized by L. V. Heilbrunn, who more recently (1926) made a series of determinations of the viscosity at different rates of shear of the protoplasm of the egg of the mollusc *Cumingia*, using the centrifuge method. He found no difference in the viscosity coefficient at different centrifugal forces, and his results have since been confirmed by the more accurate work of Howard (1932) on eggs of various sea urchins. These results have been interpreted to mean that the protoplasm of these eggs behaves as a completely inelastic fluid.

The experiments described in Table I show that there is a definite yield point to the elastic structure of the nuclear protoplasm, as shown by the low velocity of the first fall. After this first fall, however, there is no indication of any subsequent decrease in the viscosity, and, as will be shown later, no variation in viscosity with rate of shear. Heilbrunn's and Howard's experiments would readily indicate anomalous viscous flow, but could not detect a fugitive elasticity such as Table I describes, since any movement of the centrifuged particles would in its initial stages break down the elastic structure of the protoplasm, which after this breakdown might well appear inelastic. Apparently a thixotropic yield point and anomalous viscous flow need not coexist, though they may both be found, in elastic structures.

It must be admitted that these results on the nuclear protoplasm of the echi-
noderm oocyte do not necessarily apply to all types of protoplasm; it is sufficient to point out that the existence of such an elastic protoplasmic structure is not disproved by any earlier work.

The relatively rapid reorganization of the inelastic liquid phase into the elastic network of the resting nucleus is perhaps not without interest, since it implies a degree of stability, even in such fluid nuclei as these, which is not permanently disturbed by occasional streaming movements or other external mechanical causes.

As might be expected, experiments have shown that at high temperatures (30° C.) the thixotropic properties of the nuclear fluid are lost; there is no perceptible yield-point effect at those temperatures. The oocyte of *Holothuria forskali* is interesting in this connexion, since though it shows no fall of the nucleolus at ordinary temperatures, at 30° it shows a typical and rapid fall. Experiments on *Holothuria, Marthasterias* and *Echinus* show that the disorganization of the nuclear structure at these high temperatures is apparently permanent, since the thixotropy does not reappear on cooling. The oocyte of *Holothuria* after cooling from 30° continues to show a typical nucleolar fall.

C. "Polarity" of the oocyte

In the course of these experiments it was frequently found that the fall of the nucleolus was not vertical; a lateral displacement was indicated either by a movement in the photographic field, or by a change in focus of the nucleolus. The absence of any sudden velocity changes during such an observation showed that the lateral displacement was not due to any obstacle in the path of the fall, but suggested that some constant deflecting force was present. The existence of such a force acting on the nucleolus was, however, most strikingly shown by the measurements of the velocity of fall. If \( A \) and \( B \) are two points at opposite ends of a diameter of the germinal vesicle, it is usually found that the velocity of fall in the direction \( AB \) is not the same as that in the direction \( BA \), though the path traversed, and hence the geometrical relationship of the nucleolus to the nuclear boundary, is the same in the two directions. Furthermore, the difference in the two velocities is a constant one, within the limits of experimental error; the characteristic velocity of fall is repeated each time the nucleolus falls in the same direction.

Fig. 2 illustrates such an experiment involving eighteen successive falls in a single oocyte. The alternate increase and decrease of the velocity of fall is clearly shown, and the difference between the means of the velocities in the two directions is statistically significant (\( t = 6.4 \)). It must be borne in mind that the nucleolus moves with constant velocity for only a small portion of its path across the nucleus, and the limits of accuracy of the measurement of its position, even when using the photographic technique, are such that there is a considerable experimental error in the determination of any one velocity.

Table II summarizes a number of experiments on the oocytes of *Echinus esculentus*. The significance of the difference between the mean velocities in the two
Fig. 2. Velocity of fall for eighteen successive falls in a single oocyte. The velocity ordinate is measured in terms of an arbitrary unit of velocity; successive falls are equally spaced along the horizontal axis. Points with odd numbers represent falls in the opposite direction to those with even numbers. Heavy line: mean velocity of fall for even numbers; broken line: mean velocity of fall for odd numbers.

directions $AB$ and $BA$ (to use the terminology explained above), has been estimated by a calculation of the value of "Student's" factor $t$. Here

\[ t = \frac{M_1 - M_2}{\sqrt{\sigma_{M_1}^2 + \sigma_{M_2}^2 - 2\rho_{12}\sigma_{M_1}\sigma_{M_2}}} \]

$M_1$, $M_2$ = the mean velocities of fall in the two directions $AB$ and $BA$

$\sigma_{M_1}$, $\sigma_{M_2}$ = the standard errors of the two corresponding means,

$\rho_{12}$ = the correlation coefficient between successive values of $v$, taken in pairs.

This correlation coefficient is generally zero, but under conditions where the viscosity of the nuclear protoplasm is varied throughout the experiment (as by changing the temperature), the introduction of the coefficient $\rho_{12}$ enables one to eliminate the general trend of the values of $v$ due to such temperature changes.

Table II. *Ratio of velocities of fall in two opposing directions (see text)*

<table>
<thead>
<tr>
<th>Exp.</th>
<th>No. of falls observed</th>
<th>Statistical significance $t$</th>
<th>Ratio $v_1/v_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>2.0</td>
<td>1.077</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>0.6</td>
<td>1.017</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>4.4</td>
<td>1.080</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>6.2</td>
<td>1.090</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>Zero</td>
<td>1.092</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>Zr</td>
<td>1.030</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>2.1</td>
<td>1.126</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>Zero</td>
<td>1.021</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>5.5</td>
<td>1.167</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>Zero</td>
<td>1.062</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>Zero</td>
<td>1.040</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>Zero</td>
<td>1.089</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>Zero</td>
<td>1.110</td>
</tr>
<tr>
<td>14</td>
<td>11</td>
<td>Zero</td>
<td>1.002</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>1.4</td>
<td>1.047</td>
</tr>
<tr>
<td>16</td>
<td>38</td>
<td>6.1</td>
<td>1.040</td>
</tr>
</tbody>
</table>
Where the calculated value of the factor \( t \) is greater than 2.0, the velocity difference observed is considered to be statistically significant. Of the sixteen experiments listed in Table II, six give significant velocity differences, while the remaining ten fail to satisfy the statistical criterion for significance because the difference is too small for the number of falls studied. An impossibly large number of falls would be required to establish the significance of ratios of \( \frac{v_j}{v_2} \) less than 1.03.

It will be noted from a consideration of the six statistically significant results that the velocity difference observed varies considerably in the different experiments. One of the most satisfactory experiments (no. 16, where \( t = 6.1 \)) indicated a velocity difference of only 4% in the two directions, whereas another good experiment (no. 9, where \( t = 5.5 \)) showed a difference of almost 17%. The variation of the velocity difference in such different experiments is a real variation, and is not due to experimental errors.

It is a significant feature of all these experiments that whenever the difference between the two mean velocities of fall is small, the lateral deflexion shown by the nucleolus is considerable. It is not possible to estimate accurately the extent of this lateral deflexion, since it is in part shown by a motion in the plane of photography, and in part by a change in the sharpness of focus of the nucleolus (the latter occurs when the motion is parallel to the microscope axis). However, a maximum lateral velocity of the order 5-6% of the vertical velocity of fall has been observed in Exp. 8 of Table II, where the velocity difference of fall was only 2%.

### Table III. Relationship of the ratio \( \frac{v_j}{v_2} \) to the shape of the oocyte

<table>
<thead>
<tr>
<th>( \frac{v_j}{v_2} )</th>
<th>Parallel to long axis</th>
<th>Perpendicular to long axis</th>
<th>Indeterminate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>( &lt; 1.06 )</td>
<td>3 exps.</td>
<td>3 exps.</td>
<td>2 exps.</td>
<td>8 exps.</td>
</tr>
<tr>
<td>( &gt; 1.06 )</td>
<td>3 exps.</td>
<td>2 exps.</td>
<td></td>
<td>7 exps.</td>
</tr>
</tbody>
</table>

These results can all be explained if there exists some constant force acting on the nucleolus in a fixed direction relative to the oocyte. The values for the ratio \( \frac{v_j}{v_2} \) vary from 1.002 to 1.167, so that they are not related to disposition of the apparatus, or to any geometrical factor external to the oocyte. This is borne out by the actual distribution of the values of \( \frac{v_j}{v_2} \). Table III shows that the values are distributed equally about the value 1.06. (The high value of 1.167 in Exp. 9 has been eliminated from Table III, since this particular experiment was carried out over a varying range of high temperatures, and is therefore not strictly comparable with the others.) The highest value of \( \frac{v_j}{v_2} \) included in Table III is 1.126, which is close to twice the velocity difference for the mid-point of the range. It can be shown that such a distribution would be expected if we are dealing with a constant force acting along an axis with a random orientation to the vertical.

Many of the oocytes were not spherical, but elongated in shape. It seemed possible that the axis of the force might be related to the long axis of the oocyte, but Table III shows that this was not the case. The number of experiments is small,
but the figures do not support the suggestion that the velocity difference is related to the shape of the oocyte; in point of fact, the two oocytes which showed the largest velocity differences were two which most nearly approached the spherical shape.

Child (1915) has pointed out that the axis of developmental polarity of the annelid egg is determined by the point of attachment of the oocyte to the ovarian tissue, and is also indicated by an eccentricity of the nucleus. The present experiments show no relation between the axis of the force and the nuclear eccentricity for the *Echinus* oocyte, though it must be admitted that the oocytes are frequently much distorted in being expressed from the animal, so that evidence based on oocyte shape or nuclear eccentricity is probably quite unreliable.

It may be of interest to mention here that the nucleus of the *Echinus* oocyte is usually spherical; only those oocytes which possessed spherical nuclei were used for the present experiments. We may therefore conclude that the polarity of the oocyte is not determined by any visible asymmetry in the system.

The magnitude of the force on the nucleolus necessary to produce the observed difference in velocity may readily be calculated. The force will be such as to produce on a stationary nucleolus in the centre of the nucleus a velocity of approximately 6% of the normal velocity of fall. The value of 6% is one-half of the maximum observed velocity difference (neglecting the extreme case, as previously explained), since in one direction the force accelerates the velocity of fall, and in the opposite direction retards it. As the observed velocity of fall is 0.65 µ per sec. at 14° C., the asymmetrical force on the nucleolus is one which would produce a velocity of 0.04 µ per sec. Assuming the nuclear viscosity as 0.1 and the Ladenburg constant as 0.29, we have

\[ F = \frac{6\pi\eta u}{k} = 1.03 \times 10^{-8} \text{ dynes.} \]

**THE ORIGIN OF THE POLARITY**

The source of the asymmetrical force acting on the nucleolus during its fall is extremely difficult to determine. The experiments described in this section of the paper are mainly concerned with the elimination of various possible sources of the force, and for the most part they throw only an indirect light on the mechanism involved.

It has already been shown that the direction of the axis along which the force acts is not related to any factors external to the oocyte. This rules out all convection currents (whether due to temperature changes in the fluid surrounding the oocyte, or to specific gravity differences of nuclear constituents giving rise to streaming currents) and all other factors dependent on gravitational stimuli. It is necessary to search for possible mechanisms inside the oocyte.

The force on the nucleolus may be mechanical or electrical in origin. Mechanical forces would include (1) heterogeneity of the nuclear fluid, (2) asymmetry of the nucleolus, and (3) streaming movements in the nuclear protoplasm. These possibilities will each be considered separately.
(1) **Heterogeneity of the nuclear fluid**

Since the hydrodynamical problems of the motion of one sphere inside a spherical mass of uniform fluid have not yet been solved, it is useless to consider the theoretical implications of such a motion involving a non-uniform fluid. An attempt was made to meet this difficulty by constructing a mechanical model of the nucleus, containing a homogeneous viscous fluid, and observing the rate of fall of a model nucleolus inside it. Such a model was made from a spherical glass flask filled with treacle (Lyle’s golden syrup), containing a suitably ballasted celluloid table tennis ball. The dimensions of the two spheres were in exactly the same ratio as the diameters of the nucleolus and nucleus in the example studied in Fig. 1. The neck of the flask was completely plugged so as to produce an unbroken internal spherical surface, and the whole model was then immersed in a rectangular glass tank containing a concentrated sugar solution. The bath served to maintain a constant temperature, and at the same time eliminated refraction troubles in the photography of the model. The weight of the ball was adjusted so that the time taken to traverse the flask was the same as that required for the nucleolar fall. (In actual practice it was found more convenient to have the ball lighter in density than the syrup, so that it rose to the top of the flask. The difference, however, is immaterial to the success of the experiment, so long as the time of rise or fall is the same in the two systems.)

The model system described has one obvious limitation in the accuracy with which it can represent the oocyte nucleus. The terminal velocity of the ball is not attained within the extremely short time which we have seen is characteristic of the fall of the nucleolus, since the size of the model is about 1000 times greater than the size of the nucleus. Nevertheless, it can be shown that this does not seriously impair the comparison, and that within the limits of experimental error the velocity of fall at any instant is not different from the true terminal velocity.

Fig. 3 shows the velocity-time curve for the two systems. The velocity has been calculated as a percentage of its maximum value in each case, so that the differences in size between the two systems is eliminated in the comparison. It will be seen that the agreement between the two curves is so close that one is forced to the conclusion that the nuclear fluid is perfectly homogeneous. (Since the velocity of fall cannot be accurately determined when the nucleolus is close to the nuclear wall, it is possible that the nuclear protoplasm in this region may differ in viscosity from that in the central region. But this reservation does not affect the general argument, that regional differences in nuclear viscosity cannot be the cause of the polarity effect.)

It is significant that the two curves are in agreement over quite a wide variation in the velocity of fall. This implies that the nuclear viscosity is independent of the rate of shear—there is no anomalous viscous flow after the nuclear structure has once been broken down by an initial fall of the nucleolus. It is, of course, possible that a combination of anomalous viscous flow with a suitably varying regional difference in viscosity might reproduce the conditions of fall in a homogeneous inelastic fluid, but the balance of probability is overwhelmingly against such an interpretation.
The model system described above also permitted the value of the Ladenburg constant to be determined. This was done by comparing the rate of motion of the ball in the glass flask with its motion in a regular cylinder of syrup of known dimensions. The Ladenburg constant of a cylinder can be calculated from the formula on p. 260, and hence its value for different positions of the ball in the sphere could also be derived. When the two spheres were concentric, the value of the constant was found to be 0.29.

Further evidence for the homogeneity of the nuclear protoplasm was provided by observations on the Brownian movement of inclusions in the nuclear protoplasm, but these experiments will be dealt with later, in a consideration of the protoplasmic streaming.

![Velocity-time relationship for a single fall](figure3.png)

**Fig. 3. Velocity-time relationship for a single fall. Ordinate: velocity in percentage of maximum value. Circles from observations on nucleolus; crosses from the large mechanical model of the system.**

(2) **Asymmetry of the nucleolus**

So far as can be ascertained by microscopic examination, the nucleolus of the *Echinus* oocyte is perfectly spherical in shape. It is conceivable that there may be some superficial asymmetry, invisible by reason of its similarity of refractive index to that of the nuclear fluid, which could affect the rate of fall differently in the two directions of fall. It is also possible that the nucleolus may be attached to some of the chromosomes, as in *Drosophila* (see Dobzhansky, 1934), though the work of Manton (1935) has suggested that the chromatin in vesicular nuclei is probably peripheral in position.

All these possible sources of a polarized velocity of fall are ruled out by determinations in which the nucleolus is reoriented frequently during the experiment. If the fall is accompanied by a small amount of lateral motion of the nucleolus, then the final stage of the fall is accomplished by the rolling of the nucleolus down the lower curved surface of the nuclear membrane. Successive falls will then be characterized by a continuously changed nucleolar orientation—but the velocity difference under such circumstances persists unchanged. By rotating the whole
oocyte very slowly, it is possible to roll the nucleolus through $180^\circ$ against the wall of the nucleus. (The change in orientation can be followed by reference to the position of small vacuoles frequently contained in the nucleolus.) After such a $180^\circ$ rotation, the nucleolus can then be brought to the centre of the nucleus and allowed to fall in any definite direction.

In no case has the reorientation of the nucleolus affected the velocity of fall, or of the lateral motion during the fall, so that it can safely be assumed that the polarity cannot be ascribed to any asymmetry of the nucleolus or to its attached structures.

(3) Streaming movements of the nuclear protoplasm

Nuclear streaming movements offer perhaps the most probable of all the mechanical explanations for the phenomenon. A constant cyclosis of the nuclear protoplasm, similar to that described by the writer (Harris, 1935) in the cytoplasm of the egg of *Sabellaria*, would readily account for a constant difference in the rate of fall of the nucleolus, and also for the lateral movements observed.

This possibility has been ruled out by a study of the motion of small inclusions in the nuclear protoplasm. If the clear fluid contained in the germinal vesicle is carefully examined with a good oil-immersion lens and a suitable colour filter, there will occasionally be seen in it small particles varying in diameter from 0.5 to 3 $\mu$, which differ very slightly in their refractive index from that of the nuclear fluid. The difference in refractivity is so small that it is easy to miss them completely, but when found the smaller ones can be seen to undergo Brownian movement. In a few cases it has been possible to follow the movements of a single particle for some considerable time, though this can only be done when the eyes of the observer are completely free from all previous strain. The particles occur in all parts of the nucleus, from the centre to the nuclear wall, so that any streaming movement of the nuclear protoplasm would be readily detected. No such streaming motion has ever been seen. Furthermore, the motion of the particles is precisely that which would be expected if it were due to Brownian movement alone. This fact has been established by employing the method so successfully used by Pekarek (1930) for the determination of the viscosity of various types of protoplasm. The method, first developed by von Furth (1917), and called by him the "Methode der mittleren doppelseitigen Erstpassagezeiten", consists in timing the frequency with which the moving particle crosses the lines of a micrometer grid in the field of view. For details of the method and its limitations, the reader is referred to Pekarek's paper; it is sufficient to point out that it ultimately measures the time required for the particle to move a distance equal to that between adjacent lines on the grid, only the component perpendicular to these lines being measured.

Owing to the extreme eyestrain involved in following these almost invisible particles, it has only been possible to obtain a reliable set of results for a single oocyte, but the order of magnitude of the particle displacements has been confirmed on a number of oocytes; evidently the viscosity is fairly constant for different oocytes. Table IV shows the results obtained for the movement of four different
particles moving in the nuclear protoplasm of a single oocyte. In two cases the vertical component of the movement was followed, and in the other two the horizontal component (the directions vertical and horizontal refer merely to the field of vision, and denote two mutually perpendicular axes), and the similarity of the viscosity value from all four particles is very strong evidence against any constant protoplasmic streaming in the nucleus.

Table IV. Determination of the absolute viscosity of the nuclear fluid by observations on Brownian movement

<table>
<thead>
<tr>
<th>Particle</th>
<th>No. of crossings</th>
<th>Time taken sec.</th>
<th>Particle radius</th>
<th>Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>107</td>
<td>0.5</td>
<td>0.068</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>239</td>
<td>0.8</td>
<td>0.101</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>223</td>
<td>0.8</td>
<td>0.095</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>398</td>
<td>1.2</td>
<td>0.112</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>0.094</td>
</tr>
</tbody>
</table>

No great accuracy can be claimed for a result based on so few measurements; but the small spread of the viscosity values suggests that the mean of 0.094 is a reasonable estimate of the absolute viscosity of the nuclear protoplasm. It is interesting to compare this value with that calculated by Heilbrunn (1928) from Gray's (1927) data on the nucleolar fall. Heilbrunn assumes that the nucleolar diameter is 16 \( \mu \), its velocity of fall (taken from Gray's measurements) to be 0.4 \( \mu \) per sec., and the difference in specific gravity between the nucleolus and the nuclear fluid to be 0.1. The last figure is purely an estimate, but Heilbrunn gives reasonable evidence for its acceptance. If these figures are employed to calculate the viscosity of the nuclear fluid (using equation (3)) a figure of 0.35 is obtained. Heilbrunn's result of 0.02 appears to be erroneous. The above result is not corrected for the Ladenburg factor, which we have seen is 0.29. Introducing this, the viscosity value becomes 0.101, extremely close to that obtained above as a result of observations on the Brownian movement. If instead of Heilbrunn's figures, we substitute those obtained by the writer's photographic method, which indicates a nucleolar diameter of 20 \( \mu \), and a maximum velocity of fall of 0.65 \( \mu \) per sec., we obtain a value of 0.097, in still closer agreement with the Brownian movement observations. The closeness of the agreement is of course quite fortuitous; the error of the Brownian movement determination may well approach \( \pm 30\% \), while the other value is based on a purely hypothetical estimate of the specific gravities of the nucleolus and the nuclear fluid.

The velocity of protoplasmic streaming which would be required to produce the observed differences in the rate of nucleolar fall is about 0.04 \( \mu \) per sec. In 200 sec., the particles studied in Table IV would therefore cover a distance of about 8 \( \mu \), equal to about five divisions on the micrometer grid employed. Such a motion would have seriously affected the viscosity values obtained in Table IV. When it is also seen that the variation of displacement with particle size follows the law of
Brownian movement (protoplasmic streaming would naturally affect particles of all sizes equally), it will be seen that the evidence is strongly against the existence of any streaming movement large enough to account for the polarity.

(4) Electrical forces on the nucleolus

Since the possibility of a mechanical origin for the polarity has been eliminated, there remains to be considered the force on the nucleolus which might arise owing to the presence of a potential field.

Such a hypothesis implies that there must exist a surface charge on the nucleolus as well as an electric field of a suitable magnitude. It may be profitable at this point to determine the field strength and surface charge required to account for a force of the observed magnitude.

If the surface charge on the nucleolus is similar to that on other suspended particles, its ζ-potential may well be of the order of 15 mV. The gradient of the electric field necessary to produce on such a charged sphere a cataphoretic velocity of 0.04 μ per sec. is given by the equation

$$\psi = \frac{1}{4\pi \eta \times 300^2} \frac{HD\zeta}{\eta},$$

where \(\psi\) = the cataphoretic velocity of 0.04 x 10^{-4} cm. per sec.,
\(H\) = the field strength in volts per cm.,
\(D\) = the dielectric constant of the nuclear fluid (80 if assumed equal to that of water),
\(\zeta\) = the surface charge, assumed to be 15 mV.,
\(\eta\) = the viscosity of the nuclear fluid, assumed to be 0.1.

This gives a field strength of approximately 0.3 volts per cm., and implies that at opposite ends of the nuclear diameter there should be a potential difference of the order of 1.5 mV., which seems a reasonable magnitude for such an intracellular potential difference.

It is difficult to obtain evidence that such a system is in fact the source of the polarity. That the nucleolus should possess a surface charge is no doubt to be expected. The application of an external potential gradient to the Echinus oocyte does produce a movement of the nucleolus to the cathode. This experiment is not always successful, since the application of too high an external field often results in the gelation of the nucleus, shown by the immobilization of the nucleolus if the current is applied while it is falling. But by using currents of the order of 12 μA., the migration of the nucleolus to the cathode has been clearly demonstrated; the direction of motion changes each time the current direction is reversed. Care was taken to see that no movement of the oocyte or of the nucleus inside the oocyte took place during the experiment. It is, of course, impossible to estimate the magnitude of the potential field in which the nucleolus was moving, by reason of the complexity of the circuit—sea water, cell membrane, cytoplasm, nuclear membrane, nuclear fluid, etc.—but the experiment definitely proves the existence of a surface charge on the nucleolus. Apparently nuclear streaming does not occur as a result of the
application of the external potential field; the minute nuclear inclusions showed no sign of movement other than their normal Brownian motion.

This last result indicates that these inclusions carry no surface charge; viscosity observations based on their Brownian movement are therefore not subject to error from this source. The absence of charge on these inclusions is to be expected, since if there is a constant potential field across the nucleus, all charged particles would migrate to the nuclear membrane.

Though the experiments indicate that the nucleolus carries a surface charge, it has not yet been found possible to demonstrate the existence of the electric field. The difficulty of measuring such small potential differences in the internal protoplasm of the living cell is well known. In collaboration with Lord Rothschild, an attempt was made to observe such a potential difference, using agar-sea water microelectrodes, but the results were negative. The surface of the oocytes, however, is of such a nature that it is difficult to ensure that electrical contact has actually been made between the microneedle and the internal protoplasm, even when the tip of the electrode has penetrated deeply into the cytoplasm. Gelfan (1928a) claims to have found a potential difference between different parts of the protoplasm of a cell of Nitella, the direction of the field being determined by the direction of protoplasmic streaming in the cell. It is possible that the potential difference observed by Gelfan is merely a result of the streaming of the protoplasm past the stationary electrodes; as a demonstration of an internal potential difference the experiment is of little value. Cinematographic observations have shown (see Harris, 1935) that continuous streaming movements of the protoplasm are taking place in the cytoplasm of these oocytes, but it would certainly be unwarrantable to assume that these movements prove the presence of internal potential differences. Dorfman's (1933) demonstration of potential differences existing in the unfertilized egg of the frog is perhaps more relevant to the present discussion, but since the insertion of the electrode is probably followed by the activation of the egg, the observations are not strictly comparable with the present ones on Echinus.

If such potential differences are set up in the protoplasm of a cell by oxidation mechanisms, the polarity effect in the oocyte should be altered by the presence of respiratory inhibitors such as potassium cyanide. Such an effect has been proved to exist. If oocytes are transferred to sea water containing M/500 KCN, the force on the nucleolus disappears, and there is then no significant difference in the velocity of fall in the two opposing directions. In four such cases the mean value of the ratio $v_1/v_2$ was found to be 1.017, as opposed to the normal value of 1.06 (according to Table III). The value 1.017 was not significantly different from unity, and furthermore, no lateral motion was observed in these four experiments. By reason of the considerable experimental error involved in the measurement of any one velocity of fall, it is not feasible to determine how rapidly the effect of the KCN is produced. In one experiment, however, the mean ratio $v_1/v_2$ for the first 6 min. of the experiment was 1.04, being just significantly different from unity. After 55 min., the ratio had fallen to 0.995.

It is a well-known fact that KCN stops protoplasmic streaming. This is true for
the streaming movements in these oocytes; the cessation of streaming can readily be observed when the film is projected at the normal speed of 16 frames per sec.

It has been shown that the surface charge on the nucleolus is not appreciably altered in the cyanide solution; the migration of the nucleolus to the cathode of an external potential field takes place equally well whether KCN is present or absent.

A further factor which seems to influence the polarity is that of temperature. If during an experiment the temperature of the oocyte is raised above 30° C., the velocity difference disappears permanently, and is no longer observed even after the oocyte has regained the normal temperature. It may be added that in the short time (1 hr.) which such an experiment occupies, there is no evidence of coagulation of the nuclear fluid at these temperatures; the viscosity continues to decrease with rise in temperature up to 35° C., though the thixotropic effect disappears after such treatment.

DISCUSSION

The results presented in this account open up a wide field for speculation. But in the absence of so many experimental details which must be added before the landscape is even partially mapped out, it would seem useless to venture too far into the regions of the hypothetical in this discussion.

It is evident that the fluid nucleus of echinoderms possesses a stable, though not a permanent, structure. In a state of mechanical quiescence, it must contain a complex network which can rapidly be reformed after being destroyed by mechanical agitation. Perhaps the picture of such a resting nucleus filled with a meshwork of interlaced fibres is not so incorrect as Hardy (1899) and others have led us to believe. Chambers (1924), while stressing the complete fluidity of such nuclei, lays emphasis also on their readiness to gelate, which he attributes to their nucleic acid content. Such a rapid gelation is doubtless aided by the presence of such a meshwork, which can form a condensation centre for the gelation process.

It is a little difficult to imagine a structure of this type offering a considerable resistance to the initial passage of the nucleolus, and at the same time allowing the free and random motion of particles such as those used for the viscosity determination. The diameter of these particles is of the order of 10 % of the nucleolar diameter, yet they appear to be in true Brownian movement. If the size of the nuclear meshwork were sufficiently large to permit the free motion of such particles, it would be so large that irregularities in the motion of the nucleolus would be visible; such irregularities are not shown. The alternative is to suppose that the mesh is very fine, but that its strength is low enough to present the appearance of a fluid of viscosity ten times that of water. The destruction of the network would then correspond to a reduction in viscosity of about 25 %, as indicated by the increased velocity of nucleolar fall in Table I.

The permanent disappearance of the thixotropic property in these nuclei when heated to temperatures in the neighbourhood of 30° C. is of some interest. Loeb (1925) has shown that the fertilized ova of Strongylocentrotus purpuratus are killed
by an hour's exposure to a temperature of 32° C. One has always felt dissatisfied with theories of heat death based on such factors as protein coagulation or lipoid melting point; the present observations on protoplasmic structure may well provide a more probable, though still unexplained, answer to the problem.

Existing quite independently of this nuclear structure, since it is seen when the structure has been mechanically destroyed by the passage of the nucleolus, is the field of force which has been shown to act on the nucleolus itself. This force is considered to be electrical in nature, since no suggested mechanical explanation will fit the experimental facts. A relatively small potential difference of 1.5 mV. across the nucleus will suffice to account for the polarity, but even with this low potential there is presumably some energy dissipated in the maintenance of a constant current flow. It is therefore of some interest to estimate the magnitude of this energy output, and to compare it with the metabolism of the unfertilized egg.

Gelfan (1928b) estimated that the electrical conductivity of the protoplasm of Asterias oocytes was $26.2 \times 10^{-3}$ reciprocal ohms per cm. cube. No estimate exists for the conductivity of the nuclear fluid, but the above figure may serve as a basis for the present calculation. Assuming that the potential difference of 1.5 mV. is being applied to a cube of protoplasm of side 70 μ (the approximate diameter of the nucleus), the energy required to maintain the current flow can be calculated to be $3.6 \times 10^{-8}$ cal. per oocyte per hour. This may be compared with the oxygen consumption of the unfertilized Arbacia egg, which Whitaker (1933) has found to be 0.4 mm.³ per hour per 10 mm.³ of eggs. Assuming that the energy liberated is that provided by the complete aerobic oxidation of glucose, this gives an energy production for the volume of a single Echinus egg as $1.6 \times 10^{-6}$ cal. per hour. The maintenance of the current flow will therefore absorb only about 2% of the total available energy. The calculation involves a number of assumptions, but it will serve to show that the maintenance of such a potential field is probably well within the powers of the metabolism of the oocyte.

Judging from the sensitivity of the system to cyanide, which causes also a rapid cessation of cytoplasmic streaming, the mechanism for the maintenance of the potential field is to be found not in the nucleus, but in the cytoplasm. If so, there must be still more energy dissipated in the cytoplasm itself. It is possible that the major portion of the energy derived from the respiration of the oocyte is concerned with the maintenance of this field, and that not only the polarized force on the nucleolus, but also the normal cytoplasmic streaming movements, result from the presence of the electric field. It is perhaps worth noting that the cataphoretic velocity of particles carrying a 15 mV. ζ-potential in a field of this magnitude is approximately 2.5 μ per min., a speed which is very similar to that of the small scale streaming movements observed by the writer (Harris, 1935) in the protoplasm of unfertilized Sabellaria eggs.

Although the experiments have clearly shown that the potential field disappears irreversibly at temperatures above 30° C., it is at present not possible to state that the disappearance of the thixotropy and of the electric field are simultaneous at high temperatures. It seems possible, however, that the two phenomena are related. If
so, then the field is concerned in the maintenance of the stable nuclear structure; it is not the structure which determines the field, since the field persists even when the structure is mechanically destroyed.

It is perhaps unnecessary to point out the significance of this polarized system in the conception of axial gradients. According to Jenkinson (1911) and Lindahl (1932) the axis of polarity of the sea urchin egg is already established in the oocyte stage, and it seems probable that this axis is related to a differentiation in the metabolic activity of the opposite poles of the oocyte.

The oocytes of Echinus used in the present study are unsuitable for the determination of this biological axis of polarity; all that has been established is that the force on the nucleolus is consistent with the ideas of a metabolic gradient polarity. However, it has been possible, in two experiments with the oocytes of Marthasterias glacialis, to identify the point of attachment of the oocyte to the ovarian tissue by a small projection on the surface of the cell membrane. In both these experiments the direction of the polarized force on the nucleolus coincided with the oocyte diameter passing through this point of attachment.

Apart from the slight pressure applied to keep the oocytes in place, and the light intensity necessary to photograph them, it must be emphasized that the oocytes in these experiments were under conditions not greatly different from those of their normal life. The effect of light and pressure can probably be disregarded, since the observed axis of polarity bears no relationship to the orientation of the light source or of the pressure. In two or three experiments with the oocyte of Marthasterias glacialis, several oocytes matured in the course of the experiment. This criterion of normality is, of course, not applicable to the eggs of Echinus, since the maturation of the oocyte in this species takes place in the ovary.

SUMMARY

A study has been made of the rate of fall of the nucleolus through the nuclear fluid of echinoderm oocytes. Analysis of the results obtained on the oocytes of Echinus esculentus leads to the following conclusions:

1. The absence of sudden velocity changes during the fall indicates the absence of gross heterogeneities in the nuclear fluid.

2. The nuclear fluid is thixotropic. Complete reconstitution of its elastic structure takes place within 10 min. of its disruption by the moving nucleolus.

3. In addition to the gravitational force producing the fall, there is acting on the nucleolus a much weaker force, constant in magnitude and direction for any one oocyte.

4. The presence of this additional force is indicated by a constantly reproducible difference in the rate of nucleolar fall in two opposing directions, and also by horizontal motion of the nucleolus during its fall.

5. The force does not arise from viscosity differences in different regions of the nucleus; the nuclear fluid is apparently homogeneous and has an absolute viscosity of approximately 0.1, i.e. 10 times that of water. Neither nucleolar asymmetry nor streaming of the nuclear protoplasm can account for the phenomenon.
6. The electrical nature of the force on the nucleolus is suggested by the elimination of these possible mechanical explanations and by the presence of a surface charge on the nucleolus. The order of magnitude of the potential field required by this hypothesis is consistent with the energy output of the respiring oocyte. The polarized force on the nucleolus is abolished in M/500 potassium cyanide, and also by heating above 30° C.

7. The bearing of these results on the structure of the nucleus and on the origin of polarity in the unfertilized egg is discussed.

It is a pleasure to acknowledge the interest and help of Prof. J. Gray throughout this work. Most of the experiments were carried out at the Millport Laboratory of the Scottish Marine Biological Association, and the author is greatly indebted to Mr R. Elmhirst and his staff for the facilities and material so abundantly provided there. Part of the expenses were met by a grant from the Government Grant Committee of the Royal Society.

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